

CLAIMS:

1. An assay method for detecting a particular nucleic acid sequence in a sample comprising:
 - (a) mixing a sample containing nucleic acids with single stranded oligonucleotide probes having a sequence complementary to the particular nucleic acid sequence to be detected, said probes having a detectable label attached to the single strand and a first member of a specific binding pair attached to the single strand in a position distal to the detectable label, and incubating the mixture containing the sample and the probe under conditions wherein complementary single stranded nucleic acids hybridize and further wherein substantially all unhybridized single stranded nucleic acids in the sample are hydrolytically digested;
 - (b) subsequent to hydrolysis of single stranded nucleic acids, contacting the mixture with a support having second members of the binding pair attached thereto, such that specific binding pairs form between the first member of the specific binding pair attached to the oligonucleotide probe and the second member of the specific binding pair attached to the support, the specific binding pairs being coupled to the support; and
 - (c) separating the support and binding pairs coupled thereto from the mixture and determining detectable label coupled to the support, the amount of detectable label coupled to the support being proportional to the amount of nucleic acid having the particular sequence to be detected which was present in the sample.
2. The assay method according to claim 1, wherein the oligonucleotide probe includes ribooligonucleotides and ribonuclease (RNase)

specific for single stranded RNA is added to the mixture containing the probe in an amount sufficient to ensure hydrolysis of single stranded RNA.

3. The assay method according to claim 1, wherein the oligonucleotide probe contains deoxyribonucleotides (DNA) and S1 nuclease
5 is added to the sample containing the probe in an amount sufficient to ensure hydrolysis of single stranded DNA.

4. The assay method according to claim 1, wherein a plurality of RNA probes are added to the sample, the RNA probes having a plurality of sequences and different detectable groups attached to one end of
10 the probe, each detectable group being uniquely associated with a specific nucleotide sequence, and all probes having the same first member of a specific binding pair attached to the probe distal to the detectable label.

5. The assay method according to claim 1, wherein the detectable label is selected from the group consisting of radiolabels and
15 fluorescence labels.

6. The assay method according to claim 1, wherein the detectable label is indirectly attached to the oligonucleotide strand.

7. The assay method according to claim 1, wherein specific binding pair is selected from the group consisting of biotin-avidin,
20 biotin-streptavidin, antigen-antibody, and antibody-protein A.

8. The assay method according to claim 1, wherein the support is selected from the group consisting of plastic plates, glass plates, porous plates, porous beads, magnetic beads, and membrane filters.

9. The assay method of claim 1, further wherein a
25 hydrolytic enzyme specific for single stranded nucleic acids is added to the

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mixture of step (a) subsequent to hybridization of complementary nucleic acids to form dimeric hybrids.

10. A method for detecting a particular RNA sequence comprising:

5 (a) mixing a sample containing ribonucleic acids with an RNA probe having a sequence complementary to the sequence to be detected, said probe having a detectable label attached to its 5' end and a first member of a specific binding pair attached to its 3' end, and incubating for a time sufficient to permit hybridization of the probe sequence with a
10 complementary sequence;

(b) subsequent to said incubation, digesting all single stranded RNA in the mixture with RNase;

(c) contacting said RNase-digested mixture with a support having second members of the binding pair attached thereto, such that
15 specific binding pairs form; and

(d) separating the support from the mixture and measuring the amount of the detectable label bound to the support, the amount of bound detectable label being proportional to the amount of the sequence to be detected which was present in the sample.

20 11. The assay method of claim 10, wherein the specific binding pair is biotin-streptavidin.

12. An oligonucleotide comprising at least 10 bases, wherein a first base of the oligonucleotide is conjugated to a first fluorescent moiety and a second base of the oligonucleotide is conjugated to a second
25 fluorescent moiety, further wherein said first base and said second base are positioned so that upon illumination of said oligonucleotide with light which

excites said first fluorescent moiety, excitation energy is transferred to said second fluorescent moiety, whereupon said second fluorescent moiety fluoresces with peak fluorescence at a wavelength different from the fluorescent peak of said first fluorescent moiety.

5 13. The oligonucleotide according to claim 12, wherein said first base and said second base are separated by about 15 bases of said oligonucleotide.

14. An assay method for detecting a particular nucleic acid sequence in a sample comprising

10 (a) mixing a sample with the oligonucleotide according to claim 12, said oligonucleotide being complementary to a particular nucleotide sequence, and incubating the mixture under conditions where complementary single, stranded nucleic acids hybridize and unhybridized single stranded oligonucleotides are hydrolytically digested; and

15 (b) subsequent to the incubation measuring fluorescent transfer between said first fluorescent moiety and said second fluorescent moiety.

15. An oligonucleotide comprising
a segment complementary to a predetermined sequence;
20 one member of a specific binding pair conjugated to the 5' end of the complementary segment;
a first fluorescent moiety conjugated to the 3' end of the complementary segment;
a second fluorescent moiety conjugated to a portion of the
25 oligonucleotide which is not complementary to the predetermined sequence, wherein the second fluorescent moiety is positioned in the 3' direction from

the first fluorescent moiety at a distance from the first fluorescent moiety such that the presence of the second fluorescent moiety quenches the fluorescent signal from the first.

16. An assay method for detecting a particular protein in a sample comprising:
- a) mixing a sample containing proteins with an antibody that is conjugated to a first nucleic acid sequence under conditions where antigen-antibody complexes form;
 - b) then separating antigen-antibody complexes from unbound antibody, and adding an oligonucleotide probe according to claim 1, the oligonucleotide probe being complementary to the said first nucleotide sequence conjugated to the primary antibody with the antigen-antibody complexes, and incubating for a time sufficient to permit hybridization of complementary nucleic acid sequences to form nucleic acid duplexes; and
 - c) subsequent to said hybridization, adding to the sample a nuclease which specifically digests the probe unless the probe is part of a nucleic acid duplex.

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